Immunoglobulin A-induced neutrophil migration

van der Steen, L.P.E.

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CHAPTER 1

General introduction

The immune system

The immune system protects the body from infectious agents by a variety of effector cells and molecules. In humans, and other vertebrates, the immune system can be divided in the innate and adaptive immune system.[1] The innate immune system is immediately available to fight many different pathogens but is not antigen specific and innate responses do not lead to immunological memory. Myeloid cells are cells of the innate immune system and derived from a common myeloid progenitor in the bone marrow. They consist of granulocytes, macrophages and dendritic cells and can engulf and eliminate pathogens via the process of phagocytosis. Furthermore, cytokines, chemokines and/or antimicrobial substances, which can activate other immune cells, are released upon activation.[1] In addition, the complement system is activated, which can result in destruction of pathogens by inducing enhanced phagocytosis or via disruption of the bacterial cell membrane.[1]

The adaptive immune system, in contrast, is able to specifically recognize pathogens. Moreover, adaptive immune responses result in immunological memory, which gives a (life) long protective immunity to re-infection with the same pathogen.[1] B and T lymphocytes and natural killer (NK) cells are leukocytes derived from a common lymphoid progenitor in the bone marrow. B and T lymphocytes form the adaptive immune system, NK cells are however not antigen specific and therefore are defined as part of the innate immune system.[1] B and T cells have a unique prototypic antigen receptor. After an encounter with their specific antigen, T cells differentiate into effector T cells, while B cells differentiate into antibody-secreting plasma cells.[1]

Antibodies and Fc receptors

Antibodies (Abs) or immunoglobulins (Ig) comprise of two identical heavy chains and two identical light chains arranged into two antigen-binding domains (fragment antigen binding; Fab) and a constant domain (fragment crystallisable; Fc). The paired variable regions of the Fab arms are responsible for antigen recognition, while the Fc region mediates interaction with effector molecules such as complement proteins and Fc receptors.[2-4] In humans there are five main Ab classes, which are referred to as IgM, IgD, IgG, IgA and IgE isotypes.[5] Igs are present as soluble plasma proteins or are part of the specific antigen receptors on the cell-surface of B lymphocytes. Immature B-lymphocytes express IgM on their cell-surface, independently of antigen encounter. Mature, naïve B cells express both IgM and IgD and recognize antigens with low affinity. IgM is the first Ab that is produced in an immune response. It forms pentamers and is able to neutralize bacteria and/or bacterial products. It furthermore can activate the complement system, which serves as an immediate protection to the infected individual. Later, B cells undergo somatic hypermutation, which alters the variable region of the immunoglobulin, and leads to affinity maturation (which selects for survival of B cells with high antigen affinity). Additionally, isotype switching occurs that allows activated B cells to produce Abs of different isotypes (IgA, IgG or IgE), which results in a variety of effector functions.[1] The effector functions of Abs comprise neutralization of pathogens, activation of complement and/or activation of effector cells after binding and cross-linking of their respective Fc

receptors. Acivated B cells differentiate into either memory B cells, or into plasma cells that secrete higher-affinity and isotype-switched antibodies.[1] Antibodies are selectively distributed to different compartments of the body. IgG and monomeric IgA are the most prominent and second prevalent Ab class in human serum, respectively. Only low levels of IgE are present in serum, as it is mostly bound to the IgE Fc receptor (FcεRI) on mast cells. IgA is the dominant Ab at mucosal sites, where it is present as dimeric and secretory IgA.[1,5]

Immunoglobulin A

Appreciation and understanding of the immunological role of IgA has been hampered by the generally mild immune phenotype of IgA-deficient individuals in Western populations.[6] However, complete absence of IgA in both serum and secretions is rare, and increased levels of IgM in mucosal secretions usually take over the protective role of IgA.[2,6] Nevertheless, the absence of IgA is associated with certain diseases, like recurrent respiratory and gastrointestinal tract infections or disorders, autoimmunity, allergies and malignancies.[7,8] The absence of the IgA Fc receptor FcαRI (CD89) in mice, which are used for experimental studies, has further underappreciated the role of IgA in immunity (see also below).

In humans, two subclasses of IgA are described, namely IgA1 (Figure 1A) and IgA2 (Figure 1B).[9] IgA1 features an extended hinge due to the insertion of a duplicated stretch of amino acids and has three to six *O*-linked sugars, which are lacking in IgA2.[10] This gives IgA1 a T-shaped structure and a more extended reach, which may result in a simultaneous interaction with two antigen molecules separated by a considerable distance.[2,9,11,12] However, this extended reach is accompanied by an increased vulnerability to proteolytic attack by bacterial proteases, compared to IgA2.[10,13] This may explain why the two subclasses are not equally distributed throughout the body. In the large intestines and female genital tract where IgA is exposed to an abundance of proteolytic enzymes, the proportion of IgA2 is more than 50%, while in serum the predominant subclass is IgA1 (>85%).[14-16] In mucosal tissues IgA is produced by plasma cells in the lamina propria as dimeric molecules (dIgA) that are linked via their Fc-tails by a polypeptide called the 'J-chain' (Figure 1C). Dimeric IgA subsequently binds to the polymeric Ig receptor (pIgR) at the basolateral surface of epithelial cells, after which the ligand-receptor complex is endocytosed and transcytosed through the epithelial cells. At the apical surface pIgR is proteolytically cleaved and released into the lumen as secretory IgA (SIgA) (Figure 1D). The extracellular portion of the receptor, the secretory component (SC), remains bound to SIgA. This provides stabilization and prevents rapid breakdown in the hostile environment of the gut lumen.[2,17,18]

SIgA serves mostly as a non-inflammatory mucosal protector by forming an antiseptic coating of the mucosal wall, hereby inhibiting mucosal colonization of microorganisms, neutralizing viruses and hampering penetration of soluble antigens.[17] Thus, it provides a passive first line of defense against invading bacteria. Because SIgA is a poor opsonin and unable to fix the classical complement pathway efficiently, IgA has traditionally been considered as a non-inflammatory antibody,[19-21,19,22,23] Moreover, binding of SIgA to FcαRI

on inflammatory cells is partly hampered as a result of steric hindrance by SC, although this binding is increased when complement receptor type 3 (Mac-1, CD11b/CD18) acts as co-receptor.[24-26] The inability to trigger inflammatory reactions is an advantage in secretions that contain a multitude of commensal bacteria and/or environmental antigens, and initiation of inflammatory reactions against these innocuous particles would likely affect the integrity of mucosal surfaces.[22]

By contrast, cross-linking of FcαRI by complexed monomeric or dimeric IgA can vigorously induce inflammatory responses, including phagocytosis, antibody-dependent cellular cytotoxicity (ADCC), respiratory burst, degranulation, antigen-presentation and release of cytokines and inflammatory lipid mediators.[22,27]

The human IgA Fc receptor FcαRI

FcαRI is a member of the Fc receptor family of which three structurally distinct groups are known. The largest Fc receptor group contains extracellular 'Ig like' domains and includes leukocyte IgG receptors (FcγR), the high-affinity IgE receptor (FcεRI), FcαRI, pIgR, and the IgA/IgM receptor (Fcα/μR).[28] The second group is the MHC class I family containing the neonatal IgG Fc receptor (FcRn), whereas the C-type lectin superfamily is the third group that includes the low affinity FcεRII.[3,29]

FcαRI is expressed in humans, and orthologues have been identified in chimpanzees, macaques, cattle, horses and rats, but not in mice. The lack of an equivalent gene in mice is explained by disruption of the gene during a translocation event.[2] A main role for FcαRI in immune defense is supported by the fact that many pathogens have developed proteins that interfere with the binding site for IgA on FcαRI. The proteins are referred to as IgA-binding proteins (BPs). BPs are expressed by many strains of group A *Streptococcus* (*Streptococcus pyogenes*) and group B *Streptococcus*, both major human pathogens. Examples of IgA-BPs include proteins Arp4 and Sir22 of group A *Streptococcus* and β protein expressed by group B *Streptococcus*.[2] More recently, the superantigen-like protein SSL7 from *Staphylococcus aureus* has been shown to bind IgA.[30] By interacting with the same region on IgA Fc that is bound by FcαRI, IgA-BP can inhibit the triggering of an FcαRI-mediated respiratory burst, and other functions. Such blockade would therefore allow the bacterium to evade elimination mechanisms that would normally be elicited by IgA through interaction with FcαRI.[2]

Structure and expression of FcαRI

The FcαRI gene is located on chromosome 19 (at 19q13.4) and lies within the so-called leukocyte receptor cluster, whereas other FcR genes, such as FcγR and FcεRI genes, map on chromosome 1.[31,32] FcαRI expression is restricted to cells of the myeloid lineage, including neutrophils, eosinophils, monocytes and some subpopulations of macrophages (alveolar, tonsilar and splenic, but not on terminally differentiated macrophages in the small intestines).[33-37] FcαRI is additionally expressed on Kupffer cells and on interstitial and monocyte-derived dendritic cells.[23,38] Recently, it was demonstrated that platelets express FcαRI and that cross-linking induced downstream signaling.[39] FcαRI is not observed on mast cells or basophils.[40] The expression of FcαRI is modulated by cytokines

(depending on cell type), or binding of adaptor proteins to the intracellular domain of FcαRI, which can induce either de novo synthesis or transport from intracellular stores to the cell surface.[22,41-43] It was furthermore demonstrated that increased presence of serum IgA down-regulated FcαRI expression.[44,45]

FcαRI consists of two extracellular immunoglobulin-like domains (EC1 and EC2), a transmembrane (TM) region, and a short cytoplasmic tail. The two extracellular domains are folded with an angle of approximately 90° to each other.[27,46,47] FcαRI binds IgA in a 2:1 stoichiometry (Figure 2). The binding site of IgA for FcαRI lies at the interface of the Cα2 and Cα3 domains,[46,48,49] and comprises a central hydrophobic interface involving residues Leu(L)257 and Leu258 on a loop at the 'lower' end of Cα2, and Leu441, Ala(A)442, and Phe(F)443 on a close-lying loop Cα3. Met(M)433, Arg(R)382, and some surrounding charged residues also contribute to the binding (Figure 2, depicted in blue). The interaction site on FcαRI resides in the EC1 domain.[46,50,51] Especially residues Tyr(Y)35, Leu54, Phe56, Gly(G)84, His(H)85 and Lys(K)55 of FcαRI form the hydrophobic core of the interaction, with contributions from a number of surrounding charged residues (Figure 2, residues depicted in yellow).

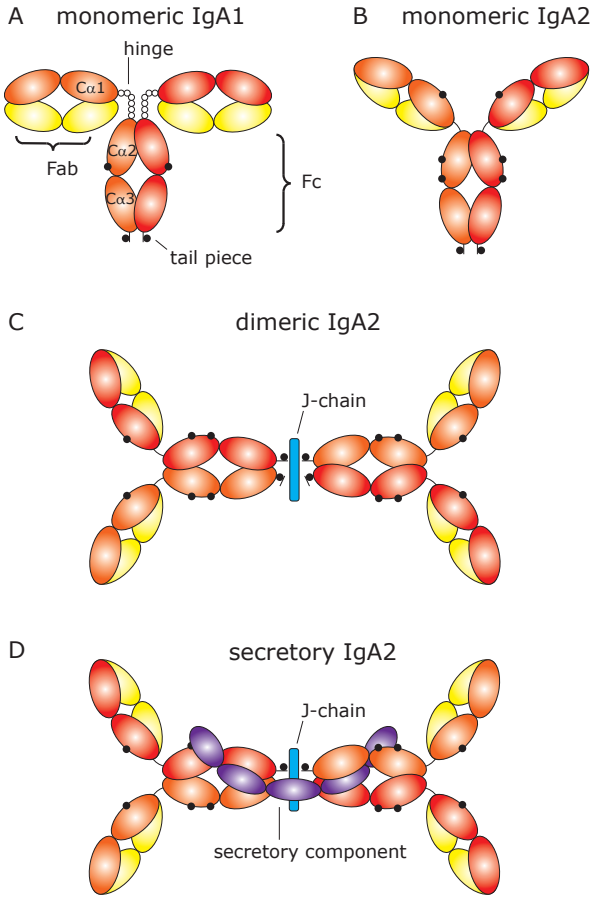

Figure 1. Schematic model of different forms of human IgA. (A) Monomeric IgA1, (B) monomeric IgA2, (C) dimeric IgA2 and (D) secretory IgA2. Heavy chains are depicted in light and dark orange, whereas light chains are shown in yellow. J-chains or secretory component (SC) are shown in blue or purple, respectively. IgA1 contains O-linked oligosaccharides in the hinge region, which are depicted as white circles, whereas N-linked oligosaccharides are shown as black circles.

For most functions, association of FcαRI with FcRγ-chain homodimer is necessary. This association occurs via the positively charged arginine 209 (R209) in the transmembrane region of FcαRI and an oppositely located negatively charged aspartic acid 11 (D11) in the TM of FcRγ-chain (Figure 2, residues depicted in red). Cross-linking of FcαRI by IgA-antigen complexes initiates immunoreceptor tyrosine-based activation motif (ITAM) dependent signaling of the FcαRI-associated FcRγ-chain.[26] *Src* kinase Lyn phosphorylates the tyrosines within the associated FcRγ-chain ITAM. These then serve as 'docking' sites for recruitment of B lymphocyte kinase (Blk), spleen tyrosine kinase (Syk), phospholipase (PLC)-γ and growth factor receptor-bound protein 2 (Grb2), which facilitates activation of multiple targets such as PI3K, PLC-γ, and components of a Grb2 containing multimolecular adapter protein complex. This signaling results in inflammatory effector functions.[26] Recently a novel pro-inflammatory role for FcαRI was identified, as cross-linking of this receptor by IgA-complexes led to release of leukotriene B4 (LTB4), which is a potent neutrophil chemoattractant. As such, a self-contained positive feedback migration loop is initiated which may result in enhanced recruitment of neutrophils until clearance of invaded pathogens is accomplished.[52]

Neutrophils

Neutrophils (or polymorphonuclear leukocytes; PMNs) are the first cells to migrate into tissues to engulf and kill microorganisms, typically at sites where the skin or mucosal barriers have been breached.[53] The first cells arrive within minutes of tissue damage and when the wound is infected, this number increases significantly.[54] The essential role of neutrophils in host defense is illustrated by the profound susceptibility to bacterial and fungal infections that results from neutropenia or defects in neutrophil trafficking.[55,56] The production of neutrophils is extensive in steady state with 1-2 x 10^{11} cells being generated per day in a normal adult human.[57] They are thereby the most abundant leukocytes in human blood, but are not present in normal healthy tissues.[1,57]

Granules, the hallmark of granulocytes (including neutrophils, eosinophils and basophils) are stores of proteins that can kill microbes.[57] Neutrophil granules are classified into three distinct subsets based on the presence of characteristic granule proteins, such as primary (azurophil) granules containing myeloperoxidase (MPO), defensins, lysozyme, elastase and cathepsin G. Secondary (specific) granules contain lactoferrin, lysozyme and FcγRII (CD32), whereas tertiary (gelatinase) granules contain gelatinase and lysozyme.[57,58] In addition to granules, neutrophils have secretory vesicles for the storage of membrane proteins, including CD11b/CD18 (Mac-1, CR3), receptors for formylmethionyl-leucyl-phenylalanine (fMLP) and lipopolysaccharide (LPS) and the Fc receptors FcγRIII (CD16) and FcαRI.[43,59,60,61] These vesicles incorporate their membrane into the surface membrane of neutrophils in response to chemotactic stimuli and stored membrane proteins are fully integrated into the surface during transendothelial migration of neutrophils.[59,62]

Neutrophil activation

When microorganisms have successfully overcome the physical barriers provided by the skin and mucus membranes and gained access to tissues, signals generated by microbes

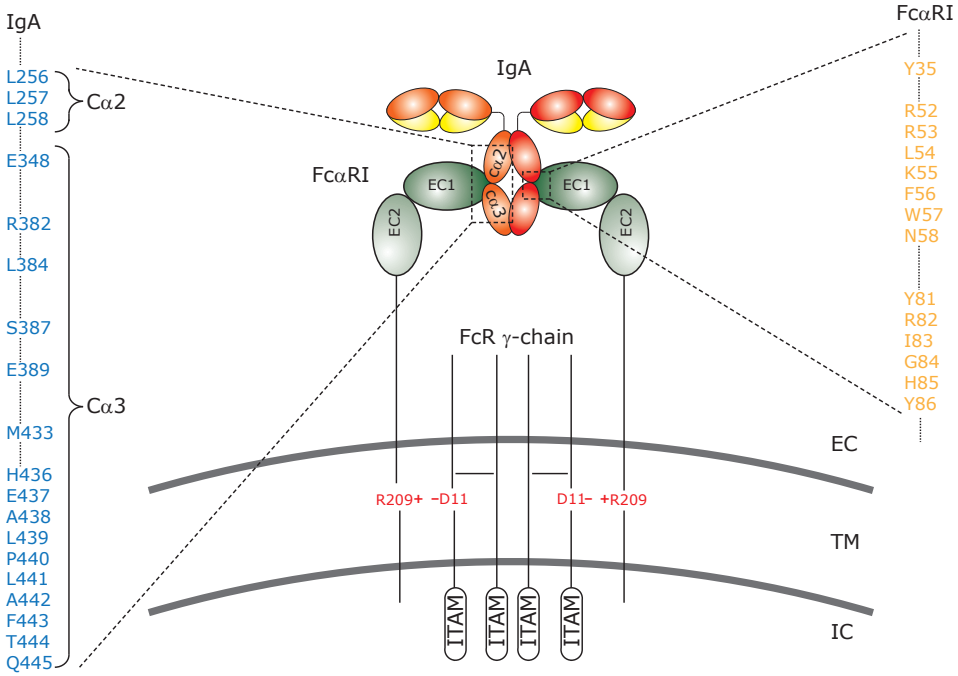

Figure 2. Schematic representation of the FcαRI–FcRγ-chain complex, binding IgA in a 2:1 stoichiometry. Two FcαRI bind each IgA-Fc part at the Cα2 and Cα3 junction via extracellular (EC) 1. Amino acids (aa) involved in ligand-receptor binding are depicted in blue for aa in Cα2 and Cα3 of IgA (left part of figure) and in yellow for FcαRI–EC1 (right part of figure). Amino acids involved in complex formation in the transmembrane regions (TMs) of FcαRI and FcRγ-chain homodimer, are depicted in red. IC; intracellular

(such as LPS and fMLP) and resident macrophages (like tumor necrosis factor (TNF)-α and interleukin (IL)-1) activate local endothelial cells. They subsequently start producing the chemokine (C-X-C motif) ligand (CXCL) 8 (or IL-8) and macrophage inflammatory protein (MIP)-2, hereby activating neutrophils and inducing rolling, firm adhesion and transcellular migration across the endothelial cell lining.[57,60] Important chemotactic stimuli for neutrophils in the interstitial space are bacterial components such as LPS and fMLP, complement factors, lipid mediators such as platelet-activating factor and LTB4, and chemokine IL-8.[82,63] During this process, chemoattractants bind to their respective neutrophil receptors, which initiates a signalling cascade.[60] Simultaneously, pattern-recognition receptors (PRRs) are activated through recognition of specific non-self patterns present on many microbes (pathogen associated molecular patterns; PAMPs).[64] Neutrophils can furthermore bind antibody-opsonised antigens via Fc receptors.[65] Neutrophils constitutively express FcαRI as well as the low to intermediate affinity receptors FcγRIIIb and FcγRIIa.[66] FcγRIIIb is a glycosyl-phosphatidyl-inositol-anchored receptor, and its signaling pathway is still not completely characterized. FcγRIIa bears an ITAM in its cytoplasmic tail that initiates signaling pathways.[67] The high affinity Fc receptor for IgG, FcγRI (CD64) is upregulated in the presence of interferon (IFN)-γ or granulocyte-colony stimulating factor (G-CSF).[66] It was demonstrated that both FcγRIIIb and FcγRII are required for an optimal phagocytosis of IgG-opsonized zymosan.[68] FcαRI is very

efficient in triggering phagocytosis after cross-linking of IgA-opsonized pathogens.[52,69,70] Besides phagocytosis neutrophils use degranulation and the formation of neutrophil extracellular traps (NETs) as effector mechanisms to eliminate invading pathogens. NETs are formed after activated neutrophils release granule proteins and chromatin that together form extracellular fibers, which can trap pathogens. NETs degrade virulence factors and killing of bacteria has been demonstrated.[71] Because the cell extrudes nuclear chromatin, this mechanism results in cell death, referred to as NETosis, which is a distinct type of cell death compared to necrosis and apoptosis.[72,73] Degranulation is induced in the case of frustrated phagocytosis (when the particle is too big to be phagocytosed). The antimicrobial contents of neutrophil granules are released into the environment via exocytosis.[74,75,60] After activation of neutrophils, downstream molecules induce assembly of the oxidative burst machinery.[57] The NAPDH oxidase complex is assembled on the cellular membrane and during the respiratory burst, this multi-component enzyme creates various reactive oxygen species (ROS), including O_2^-(superoxide), ·HO (hydroxyl radical),$^1O_2$ (singlet oxygen) and H_2O_2 (hydrogen peroxide). H_2O_2 in turn is converted by MPO into the potent oxidant hypercloric acid (HOCl).[76,77] Release of anti-microbial products by neutrophils efficiently kills pathogens, but can also lead to serious collateral damage to normal host cells.[78,79]

Exuberant neutrophil recruitment and indiscriminate release of toxic mediators contribute to the pathogenesis of many diseases. In particular, many acute and chronic diseases of the liver are mediated and/or exacerbated by neutrophilic inflammation, such as sepsis and endotoxemia, ischemia/reperfusion injury, and alcoholic, viral, and autoimmune hepatitis.[54] Additionally, IgA-induced neutrophil migration induces tissue damage in the chronic skin blistering disorder linear IgA bullous disease (LABD, see also below).[80] It is therefore essential that activation is tightly regulated and that neutrophils are removed from the tissue as soon as the infection is cleared.[77,78]

Crosstalk of neutrophils with other cells of the innate and adaptive immune system

In contrast to the general believe of neutrophils as end-stage cells without the capacity for biosynthesis, it is now clear that neutrophils can release a plethora of cytokines and chemokines that activate or attract other cells of the immune system.[79] For instance, crosstalk between neutrophils and monocyte/macrophages has been demonstrated. Resident macrophages are the first cells to detect invading pathogens. This leads to the production of pro-inflammatory cytokines and chemokines (like TNFα, IL-6, IL-8, CXCL1, CXCL2), thereby promoting the recruitment of neutrophils.[81-84] Moreover, macrophages release survival signals that increase the life-span of neutrophils, like IL-1β, granulocyte-monocyte (GM)-CSF and G-SCF.[81,85] In turn, neutrophils release classical monocyte chemokines such as chemokine (C-C motif) ligand (CCL) 2 (or monocyte chemoattractant protein-1; MCP-1), CCL3 (MIP-1α), CCL20 (MIP-3α), and CCL19 (MIP-3β), to promote recruitment of monocytes to the site of infection.[60] After pathogen clearance, apoptotic neutrophils release lipid mediators, proteins and nucleotides that act on macrophages to promote phagocytosis and removal of dead neutrophils.[81] Uptake of apoptotic neutrophils induces an anti-inflammatory phenotype in macrophages, eventually promoting

restoration of homeostasis.[86]

Crosstalk between neutrophils and dendritic cells (DCs) has been described as well. Cytokines that are released by activated neutrophils, including CCL3, CCL4, CCL5, and CCL20, recruit immature bone-marrow derived DCs from the circulation and upregulate the immunostimulatory maturation of these cells.[87,88] It has been shown that during microbial infection, neutrophils induce DC activation leading in turn to T helper 1 (Th1) cell activation. It was suggested that this effect is mediated by the interaction between DC-SIGN and Mac-1 on DCs and neutrophils, respectively.[88,89] Moreover, antigen transfer from living and apoptotic neutrophils to DCs via cell-cell contact is reported, resulting in antigen specific T cell responses.[90] Additionally, the crucial role of neutrophils in DC activation was recently confirmed using anti-Ly6G antibody depletion. In *Mycobacterium tuberculosis* infection, timely trafficking of DCs to lymph nodes and activation of $CD4^+$ T cells were both dependent on neutrophils. Furthermore, this study demonstrated that DCs presented bacterial antigens when they ingested infected neutrophils just as efficiently as they did via direct uptake of *Mycobacterium*.[91] A subpopulation of activated neutrophils constitutively expressed CC-chemokine receptor 7 (CCR7) and migrated from the periphery to the draining lymph nodes.[92] These neutrophils entered the draining lymph nodes rapidly and remained for a period of several hours, localizing mainly to the marginal sinus and superficial cortex. There they could establish brief contact with DCs and macrophages and compete for antigens.[92] Additionally, it was demonstrated that antigen cross-presentation by neutrophils stimulated cytokine production and effector functions by naïve $CD8^+$ T cells.[93,94] Moreover, crosstalk between neutrophils and T helper 17 (Th17) cells was reported as Th17 cells secrete IL-8 and neutrophils in turn attract Th17 cells via the release of CCL2 and CCL20 that bind to the receptors CCR2 and CCR6 on Th17 cells. Additionally, IL-17 upregulates the production of G-CSF by epithelial cells, which stimulates the production of neutrophils.[95]

It was furthermore demonstrated that neutrophils and NK cells interact and regulate each other's activity. NK cells promote survival of neutrophils via the release of IFNγ and GM-CSF, whereas neutrophils induce NK cell activation through release of Cathepsin G, Azurocidin, defensins and lactoferrin.[96,97] Neutrophils additionally produce B cell activating factor (BAFF) and a proliferation-inducing ligand, (APRIL, CD256). The latter was demonstrated to promote survival of plasma cells of mucosal associated lymphoid tissue (MALT).[98,99]

Diseases associated with deranged IgA-induced neutrophil activation

Several chronic inflammatory diseases are considered to be mediated by neutrophils. For instance, LABD is an autoimmune blistering skin disease, which is characterized by aberrant deposits of IgA auto-antibodies, dense inflammatory infiltrates that are dominated by neutrophils, and epidermal blisters.[100-102] IgA autoantibodies in LABD are directed against collagen XVII (BP180), which is a transmembraneous hemi-desmosomal protein involved in maintaining cell-matrix adhesion at the basement membrane of the skin.[100,102-104] Cross-linking of FcαRI by complexed IgA autoantibodies of LABD patients induced neutrophil recruitment and tissue damage, which was dependent on FcαRI.[80] This

supports that IgA-induced tissue damage by neutrophils might be prevented by blocking FcαRI.

Moreover, the same mechanism may play a role in other diseases. Since IgA is abundantly present in mucosal tissues, and neutrophils of ulcerative colitis (UC) patients take up IgA-complexes in the lamina propria, deranged IgA-induced neutrophil recruitment might also play a role in the pathogenesis of UC.[52] UC is, together with Crohn's disease (CD), a subtype of inflammatory bowel disease (IBD). The onset of UC and CD is a combination of genetic susceptibility and environmental factors.[105,106] UC is characterised by a diffuse and superficial mucosal inflammation that extends proximally from the rectum to a varying degree. Histopathological features include the presence of a significant number of neutrophils within the lamina propria and crypts, where they form microabcesses.[107] CD is not considered to be a neutrophil mediated disease, since it is characterised by compact aggregations of mononuclear cells that frequently form non-caseating granulomas.[107] The disease can manifest itself as patchy and segmented areas of transmural inflammation throughout the whole gastrointestinal tract, although the terminal ileum is commonly involved and early lesions occur mostly over Peyer's patches. At present, no cure exists for IBD, and medication is aimed to achieve remission of symptoms and prevention of relapses of the disease. Therapeutically, UC and CD are regarded as autoimmune diseases and the medication of choice consists of anti-inflammatory drugs and immunosuppressants.[105] Monoclonal antibody therapies using anti-TNFα agent Infliximab was proven efficient in induction and maintenance of remission of CD. Additionally, it was demonstrated that UC patients treated with Infliximab were more likely to have a clinical response compared to placebo.[108,109] In severe cases of UC and CD, colectomy is performed, which in some cases can cure the disease, but at high cost, as it will diminish quality of life for these patients.

OUTLINE OF THIS THESIS

In this thesis my aim is to unravel the role of FcαRI in the pathogenesis of IgA-induced chronic diseases. I hypothesize that abnormal accumulation of IgA-antigen complexes will lead to excessive cross-linking of FcαRI, which results in continuous neutrophil activation and infiltration. Ultimately, this will lead to serious tissue damage and aggravation of these diseases. Moreover, I propose that blocking IgA-FcαRI interactions may prevent tissue damage and may lead to new therapeutics for IgA-induced chronic diseases. In **chapter 2**, we describe a novel pro-inflammatory role for IgA, as cross-linking of FcαRI by IgA-coated beads resulted in release of LTB4, which is a potent neutrophil chemoattractant. Consequently, a self-contained positive feedback loop can be initiated, which results in enhanced recruitment of neutrophils until clearance of invaded pathogens is achieved. In **chapter 3**, we demonstrated that IgA-induced neutrophil migration is deranged in patients with aberrant IgA-auto-antibodies in their skin. We used serum of LABD patients (containing anti-collagen XVII IgA auto-antibodies) to show that IgA-induced neutrophil migration and tissue damage was dependent on FcαRI. In **chapter 4**, we developed a novel mouse model for LABD. Mouse transgenic for FcαRI were crossbred with mice expressing Lys/EGFP on neutrophils, hereby providing the opportunity to analyse neutrophil migration with intravital imaging. We demonstrated that anti-mouse

collagen XVII IgA antibodies injected in the ears of these mice induced extensive neutrophil migration into the interstitial space. We furthermore showed accumulation of neutrophils in blood vessels, followed by rolling and extravasation. The most commonly used therapies for IgA-induced blistering diseases are based on general immune-suppression with concomitant side effects. In **chapter 5**, we aim to develop specific, topical therapies using peptides as protein mimics that block IgA-FcαRI interactions. In **chapter 6**, we used our novel humanized IgA/FcαRI mouse model, in which human IgA is produced and neutrophils express FcαRI, to analyse the role of IgA-FcαRI interaction in the pathogenesis of experimental colitis. The different immune cell populations that may contribute to immune responses during colitis were analysed in **chapter 7**. Additionally, we analysed the presence of different cytokines that are able to activate and attract cells of the adaptive immune system and linked these to the cytokine expression profile of activated neutrophils. Finally, in **chapter 8**, I have summarized and discussed all previous chapters in relation to the current state of the art. Furthermore, I provide suggestions for future research and perspectives of possible therapeutic interventions.

REFERENCES

1. Murphy, K., Travers, P. & Walport, M. *Janeway's Immunobiology*, (Garland Science, New York, 2007).
2. Woof, J.M. & Kerr, M.A. The function of immunoglobulin A in immunity. *J Pathol* **208**, 270-282 (2006).
3. Raghavan, M. & Bjorkman, P.J. Fc receptors and their interactions with immunoglobulins. *Annu Rev Cell Dev Biol* **12**, 181-220 (1996).
4. Bruhns, P. Properties of mouse and human IgG receptors and their contribution to disease models. *Blood* **119**, 5640-5649 (2012).
5. Schroeder, H.W., Jr. & Cavacini, L. Structure and function of immunoglobulins. J *Allergy Clin Immunol* **125**, S41-52 (2010).
6. Kilian, M., Mestecky, J. & Russell, M.W. Defense mechanisms involving Fc-dependent functions of immunoglobulin A and their subversion by bacterial immunoglobulin A proteases. *Microbiol Rev* **52**, 296-303 (1988).
7. Yel, L. Selective IgA deficiency. *J Clin Immunol* **30**, 10-16 (2010).
8. Wang, N., et al. Selective IgA deficiency in autoimmune diseases. *Mol Med* **17**, 1383-1396 (2011).
9. Rifai, A., Fadden, K., Morrison, S.L. & Chintalacharuvu, K.R. The N-glycans determine the differential blood clearance and hepatic uptake of human immunoglobulin (Ig)A1 and IgA2 isotypes. *J Exp Med* **191**, 2171-2182 (2000).
10. Woof, J.M. & Kerr, M.A. IgA function--variations on a theme. *Immunology* **113**, 175-177 (2004).
11. Tarelli, E., Smith, A.C., Hendry, B.M., Challacombe, S.J. & Pouria, S. Human serum IgA1 is substituted with up to six O-glycans as shown by matrix assisted laser desorption ionisation time-of-flight mass spectrometry. *Carbohydr Res* **339**, 2329-2335 (2004).
12. Mattu, T.S., *et al*. The glycosylation and structure of human serum IgA1, Fab, and Fc regions and the role of N-glycosylation on Fc alpha receptor interactions. J *Biol Chem* **273**, 2260-2272 (1998).
13. Kilian, M., Reinholdt, J., Lomholt, H., Poulsen, K. & Frandsen, E.V. Biological significance of IgA1 proteases in bacterial colonization and pathogenesis: critical evaluation of experimental evidence. *Apmis* **104**, 321-338 (1996).
14. Brown, T.A., Murphy, B.R., Radl, J., Haaijman, J.J. & Mestecky, J. Subclass distribution and molecular form of immunoglobulin A hemagglutinin antibodies in sera and nasal secretions after experimental secondary infection with influenza A virus in humans. *J Clin Microbiol* **22**, 259-264 (1985).
15. Crago, S.S., *et al*. Distribution of IgA1-, IgA2-, and J chain-containing cells in human tissues. *J Immunol* **132**, 16-18 (1984).
16. Moro, I., Umemura, S., Crago, S.S. & Mestecky, J. Immunohistochemical distribution of immunoglobulins, lactoferrin, and lysozyme in human minor salivary glands. *J Oral Pathol* **13**, 97-104 (1984).
17. Brandtzaeg, P. Role of secretory antibodies in the defence against infections. *Int J Med Microbiol* **293**, 3-15 (2003).
18. Mostov, K.E. Transepithelial transport of immunoglobulins. *Annu Rev Immunol* **12**, 63-84 (1994).
19. Brandtzaeg, P. Update on mucosal immunoglobulin A in gastrointestinal disease. *Curr Opin Gastroenterol* **26**, 554-563 (2010).
20. Russell, M.W., Sibley, D.A., Nikolova, E.B., Tomana, M. & Mestecky, J. IgA antibody as a non-inflammatory regulator of immunity. *Biochem Soc Trans* **25**, 466-470 (1997).
21. Van Epps, D.E. & Williams, R.C., Jr. Suppression of leukocyte chemotaxis by human IgA myeloma components. *J Exp Med* **144**, 1227-1242 (1976).
22. Monteiro, R.C. & Van De Winkel, J.G. IgA Fc receptors. *Annu Rev Immunol* **21**, 177-204 (2003).
23. van Egmond, M., *et al*. FcalphaRI-positive liver Kupffer cells: reappraisal of the function of immunoglobulin A in immunity. *Nat Med* **6**, 680-685 (2000).
24. Herr, A.B., White, C.L., Milburn, C., Wu, C. & Bjorkman, P.J. Bivalent binding of IgA1 to FcalphaRI suggests a mechanism for cytokine activation of IgA phagocytosis. *J Mol Biol* **327**, 645-657 (2003).
25. Woof, J.M. & Burton, D.R. Human antibody-Fc receptor interactions illuminated by crystal structures. *Nat Rev Immunol* **4**, 89-99 (2004).
26. van Spriel, A.B., *et al*. Mac-1 (CD11b/CD18) is essential for Fc receptor-mediated neutrophil cytotoxicity and immunologic synapse formation. *Blood* **97**, 2478-2486 (2001).
27. Bakema, J.E. & van Egmond, M. The human immunoglobulin A Fc receptor FcalphaRI: a multifaceted regulator of mucosal immunity. *Mucosal Immunol* **4**, 612-624 (2011).
28. Daeron, M. Fc receptor biology. *Annu Rev Immunol* **15**, 203-234 (1997).
29. Kijimoto-Ochiai, S. CD23 (the low-affinity IgE receptor) as a C-type lectin: a multidomain and multifunctional molecule. *Cell Mol Life Sci* **59**, 648-664 (2002).
30. Langley, R., *et al*. The staphylococcal

superantigen-like protein 7 binds IgA and complement C5 and inhibits IgA-Fc alpha RI binding and serum killing of bacteria. *J Immunol* **174**, 2926-2933 (2005).
31. Kremer, E.J., *et al*. The gene for the human IgA Fc receptor maps to 19q13.4. *Hum Genet* **89**, 107-108 (1992).
32. Martin, A.M., Kulski, J.K., Witt, C., Pontarotti, P. & Christiansen, F.T. Leukocyte Ig-like receptor complex (LRC) in mice and men. *Trends Immunol* **23**, 81-88 (2002).
33. Sibille, Y., *et al*. Surface IgA and Fc-alpha receptors on human alveolar macrophages from normal subjects and from patients with sarcoidosis. *Am Rev Respir Dis* **139**, 740-747 (1989).
34. Monteiro, R.C., Kubagawa, H. & Cooper, M.D. Cellular distribution, regulation, and biochemical nature of an Fc alpha receptor in humans. *J Exp Med* **171**, 597-613 (1990).
35. Monteiro, R.C., Cooper, M.D. & Kubagawa, H. Molecular heterogeneity of Fc alpha receptors detected by receptor-specific monoclonal antibodies. *J Immunol* **148**, 1764-1770 (1992).
36. Monteiro, R.C., *et al*. Definition of immunoglobulin A receptors on eosinophils and their enhanced expression in allergic individuals. *J Clin Invest* **92**, 1681-1685 (1993).
37. Hamre, R., Farstad, I.N., Brandtzaeg, P. & Morton, H.C. Expression and modulation of the human immunoglobulin A Fc receptor (CD89) and the FcR gamma chain on myeloid cells in blood and tissue. *Scand J Immunol* **57**, 506-516 (2003).
38. Geissmann, F., *et al*. A subset of human dendritic cells expresses IgA Fc receptor (CD89), which mediates internalization and activation upon cross-linking by IgA complexes. *J Immunol* **166**, 346-352 (2001).
39. Qian, K., et al. Functional expression of IgA receptor FcalphaRI on human platelets. *J Leukoc Biol* **84**, 1492-1500 (2008).
40. Voehringer, D. Protective and pathological roles of mast cells and basophils. *Nat Rev Immunol* **13**, 362-75 (2013).
41. Bakema, J.E., *et al*. c-Jun activating binding protein 1 binds to the IgA receptor and modulates protein levels of FcalphaRI and FcRgamma-chain. *Eur J Immunol* **40**, 2035-2040 (2010).
42. Hostoffer, R.W., Krukovets, I. & Berger, M. Increased Fc alpha R expression and IgA-mediated function on neutrophils induced by chemoattractants. *J Immunol* **150**, 4532-4540 (1993).
43. Yin, N., Peng, M., Xing, Y. & Zhang, W. Intracellular pools of FcalphaR (CD89) in human neutrophils are localized in tertiary granules and secretory vesicles, and two FcalphaR isoforms are found in tertiary granules. *J Leukoc Biol* **82**, 551-558 (2007).
44. Grossetete, B., Viard, J.P., Lehuen, A., Bach, J.F. & Monteiro, R.C. Impaired Fc alpha receptor expression is linked to increased immunoglobulin A levels and disease progression in HIV-1-infected patients. *Aids* **9**, 229-234 (1995).
45. Grossetete, B., *et al*. Down-regulation of Fc alpha receptors on blood cells of IgA nephropathy patients: evidence for a negative regulatory role of serum IgA. *Kidney Int* **53**, 1321-1335 (1998).
46. Herr, A.B., Ballister, E.R. & Bjorkman, P.J. Insights into IgA-mediated immune responses from the crystal structures of human FcalphaRI and its complex with IgA1-Fc. *Nature* **423**, 614-620 (2003).
47. Maliszewski, C.R., March, C.J., Schoenborn, M.A., Gimpel, S. & Shen, L. Expression cloning of a human Fc receptor for IgA. *J Exp Med* **172**, 1665-1672 (1990).
48. Carayannopoulos, L., Hexham, J.M. & Capra, J.D. Localization of the binding site for the monocyte immunoglobulin (Ig) A-Fc receptor (CD89) to the domain boundary between Calpha2 and Calpha3 in human IgA1. *J Exp Med* **183**, 1579-1586 (1996).
49. Pleass, R.J., Dunlop, J.I., Anderson, C.M. & Woof, J.M. Identification of residues in the CH2/CH3 domain interface of IgA essential for interaction with the human fcalpha receptor (FcalphaR) CD89. *J Biol Chem* **274**, 23508-23514 (1999).
50. Wines, B.D., *et al*. Identification of residues in the first domain of human Fc alpha receptor essential for interaction with IgA. *J Immunol* **162**, 2146-2153 (1999).
51. Wines, B.D., Sardjono, C.T., Trist, H.H., Lay, C.S. & Hogarth, P.M. The interaction of Fc alpha RI with IgA and its implications for ligand binding by immunoreceptors of the leukocyte receptor cluster. *J Immunol* **166**, 1781-1789 (2001).
52. van der Steen, L., *et al*. Immunoglobulin A: Fc(alpha)RI interactions induce neutrophil migration through release of leukotriene B4. *Gastroenterology* **137**, 2018-2029 (2009).
53. Borregaard, N., Theilgaard-Monch, K., Cowland, J.B., Stahle, M. & Sorensen, O.E. Neutrophils and keratinocytes in innate immunity-cooperative actions to provide antimicrobial defense at the right time and place. *J Leukoc Biol* **77**, 439-443 (2005).
54. Kim, M.H., *et al*. Dynamics of neutrophil infiltration during cutaneous wound healing and infection using fluorescence imaging. *J Invest Dermatol* **128**, 1812-1820 (2008).
55. Lekstrom-Himes, J.A. & Gallin, J.I. Immunodeficiency diseases caused by

defects in phagocytes. *N Engl J Med* **343**, 1703-1714 (2000).
56. Kubes, P. & Mehal, W.Z. Sterile inflammation in the liver. *Gastroenterology* **143**, 1158-1172 (2012).
57. Borregaard, N. Neutrophils, from marrow to microbes. *Immunity* **33**, 657-670 (2010).
58. Galligan, C. & Yoshimura, T. Phenotypic and functional changes of cytokine-activated neutrophils. *Chem Immunol Allergy* **83**, 24-44 (2003).
59. Borregaard, N., *et al*. Stimulus-dependent secretion of plasma proteins from human neutrophils. J Clin Invest 90, 86-96 (1992).
60. Amulic, B., Cazalet, C., Hayes, G.L., Metzler, K.D. & Zychlinsky, A. Neutrophil Function: From Mechanisms to Disease. *Annu Rev Immunol* **30**, 459-89 (2012).
61. Faurschou, M. & Borregaard, N. Neutrophil granules and secretory vesicles in inflammation. *Microbes Infect* **5**, 1317-1327 (2003).
62. Sengelov, H., *et al*. Mobilization of granules and secretory vesicles during in vivo exudation of human neutrophils. *J Immunol* **154**, 4157-4165 (1995).
63. Parent, C.A. Making all the right moves: chemotaxis in neutrophils and Dictyostelium. *Curr Opin Cell Biol* **16**, 4-13 (2004).
64. Sabroe, I., Dower, S.K. & Whyte, M.K. The role of Toll-like receptors in the regulation of neutrophil migration, activation, and apoptosis. *Clin Infect Dis* **41** Suppl 7, S421-426 (2005).
65. Nimmerjahn, F. & Ravetch, J.V. Fcgamma receptors as regulators of immune responses. *Nat Rev Immunol* **8**, 34-47 (2008).
66. Ravetch, J.V. & Bolland, S. IgG Fc receptors. *Annu Rev Immunol* **19**, 275-290 (2001).
67. Van den Herik-Oudijk, I.E., Capel, P.J., van der Bruggen, T. & Van de Winkel, J.G. Identification of signaling motifs within human Fc gamma RIIa and Fc gamma RIIb isoforms. *Blood* **85**, 2202-2211 (1995).
68. Marois, L., Pare, G., Vaillancourt, M., Rollet-Labelle, E. & Naccache, P.H. Fc gammaRIIIb triggers raft-dependent calcium influx in IgG-mediated responses in human neutrophils. *J Biol Chem* **286**, 3509-3519 (2011).
69. van der Pol, W., Vidarsson, G., Vile, H.A., van de Winkel, J.G. & Rodriguez, M.E. Pneumococcal capsular polysaccharide-specific IgA triggers efficient neutrophil effector functions via FcalphaRI (CD89). *J Infect Dis* **182**, 1139-1145 (2000).
70. Hellwig, S.M., van Spriel, A.B., Schellekens, J.F., Mooi, F.R. & van de Winkel, J.G. Immunoglobulin A-mediated protection against Bordetella pertussis infection. *Infect Immun* **69**, 4846-4850 (2001).
71. Brinkmann, V., et al. Neutrophil extracellular traps kill bacteria. *Science* **303**, 1532-1535 (2004).
72. Fuchs, T.A., *et al*. Novel cell death program leads to neutrophil extracellular traps. *J Cell Biol* **176**, 231-241 (2007).
73. Steinberg, B.E. & Grinstein, S. Unconventional roles of the NADPH oxidase: signaling, ion homeostasis, and cell death. *Sci STKE* **2007**, pe11 (2007).
74. Borregaard, N. & Cowland, J.B. Granules of the human neutrophilic polymorphonuclear leukocyte. *Blood* **89**, 3503-3521 (1997).
75. Hager, M., Cowland, J.B. & Borregaard, N. Neutrophil granules in health and disease. J *Intern Med* **268**, 25-34 (2010).
76. Edwards, S.W. & Watson, F. The cell biology of phagocytes. *Immunol Today* **16**, 508-510 (1995).
77. Martin, P. & Leibovich, S.J. Inflammatory cells during wound repair: the good, the bad and the ugly. *Trends Cell Biol* **15**, 599-607 (2005).
78. Wines, B.D. & Hogarth, P.M. IgA receptors in health and disease. *Tissue Antigens* **68**, 103-114 (2006).
79. Mantovani, A., Cassatella, M.A., Costantini, C. & Jaillon, S. Neutrophils in the activation and regulation of innate and adaptive immunity. *Nat Rev Immunol* **11**, 519-531 (2011).
80. van der Steen, L.P., *et al*. Blocking Fcalpha receptor I on granulocytes prevents tissue damage induced by IgA autoantibodies. *J Immunol* **189**, 1594-1601 (2012).
81. Soehnlein, O. & Lindbom, L. Phagocyte partnership during the onset and resolution of inflammation. *Nat Rev Immunol* **10**, 427-439 (2010).
82. Garcia-Ramallo, E., *et al*. Resident cell chemokine expression serves as the major mechanism for leukocyte recruitment during local inflammation. *J Immunol* **169**, 6467-6473 (2002).
83. Grimm, M.C., Elsbury, S.K., Pavli, P. & Doe, W.F. Interleukin 8: cells of origin in inflammatory bowel disease. *Gut* **38**, 90-98 (1996).
84. Carre, P.C., *et al*. Increased expression of the interleukin-8 gene by alveolar macrophages in idiopathic pulmonary fibrosis. A potential mechanism for the recruitment and activation of neutrophils in lung fibrosis. *J Clin Invest* **88**, 1802-1810 (1991).
85. Kantari, C., Pederzoli-Ribeil, M. & Witko-Sarsat, V. The role of neutrophils and monocytes in innate immunity. *Contrib Microbiol* **15**, 118-146 (2008).
86. Filardy, A.A., *et al*. Proinflammatory clearance of apoptotic neutrophils induces

an IL-12(low)IL-10(high) regulatory phenotype in macrophages. *J Immunol* **185**, 2044-2050 (2010).
87. Bennouna, S., Bliss, S.K., Curiel, T.J. & Denkers, E.Y. Cross-talk in the innate immune system: neutrophils instruct recruitment and activation of dendritic cells during microbial infection. *J Immunol* **171**, 6052-6058 (2003).
88. Maffia, P.C., *et al.* Neutrophil elastase converts human immature dendritic cells into transforming growth factor-beta1-secreting cells and reduces allostimulatory ability. *Am J Pathol* **171**, 928-937 (2007).
89. van Gisbergen, K.P., Sanchez-Hernandez, M., Geijtenbeek, T.B. & van Kooyk, Y. Neutrophils mediate immune modulation of dendritic cells through glycosylation-dependent interactions between Mac-1 and DC-SIGN. *J Exp Med* **201**, 1281-1292 (2005).
90. Megiovanni, A.M., *et al*. Polymorphonuclear neutrophils deliver activation signals and antigenic molecules to dendritic cells: a new link between leukocytes upstream of T lymphocytes. *J Leukoc Biol* **79**, 977-988 (2006).
91. Blomgran, R. & Ernst, J.D. Lung neutrophils facilitate activation of naive antigen-specific CD4+ T cells during Mycobacterium tuberculosis infection. *J Immunol* **186**, 7110-7119 (2011).
92. Beauvillain, C., *et al*. CCR7 is involved in the migration of neutrophils to lymph nodes. *Blood* **117**, 1196-1204 (2011).
93. Beauvillain, C., *et a*l. Neutrophils efficiently cross-prime naive T cells in vivo. *Blood* **110**, 2965-2973 (2007).
94. Hufford, M.M., *et al*. Influenza-infected neutrophils within the infected lungs act as antigen presenting cells for anti-viral CD8(+) T cells. *PLoS One* **7**, e46581 (2012).
95. Pelletier, M., *et al*. Evidence for a cross-talk between human neutrophils and Th17 cells. *Blood* **115**, 335-43 (2010).
96. Costantini, C., *et al*. Neutrophil activation and survival are modulated by interaction with NK cells. *Int Immunol* **22**, 827-838 (2010).
97. Costantini, C. & Cassatella, M.A. The defensive alliance between neutrophils and NK cells as a novel arm of innate immunity. *J Leukoc Biol* **89**, 221-233 (2011).
98. Huard, B., *et al*. APRIL secreted by neutrophils binds to heparan sulfate proteoglycans to create plasma cell niches in human mucosa. *J Clin Invest* **118**, 2887-2895 (2008).
99. Scapini, P., Bazzoni, F. & Cassatella, M.A. Regulation of B-cell-activating factor (BAFF)/B lymphocyte stimulator (BLyS) expression in human neutrophils. *Immunol Lett* **116**, 1-6 (2008).
100. Schmidt, E. & Zillikens, D. Pemphigoid diseases. *Lancet* **381**, 320-332 (2013).
101. Tsuruta, D., et al. IgA pemphigus. *Clin Dermatol* **29**, 437-442 (2011).
102. Mihai, S. & Sitaru, C. Immunopathology and molecular diagnosis of autoimmune bullous diseases. *J Cell Mol Med* **11**, 462-481 (2007).
103. Sitaru, C., *et al*. Induction of dermal-epidermal separation in mice by passive transfer of antibodies specific to type VII collagen. *J Clin Invest* **115**, 870-878 (2005).
104. Sardy, M., Karpati, S., Merkl, B., Paulsson, M. & Smyth, N. Epidermal transglutaminase (TGase 3) is the autoantigen of dermatitis herpetiformis. *J Exp Med* **195**, 747-757 (2002).
105. Lichtenstein, G.R., Hanauer, S.B. & Sandborn, W.J. Management of Crohn's disease in adults. *Am J Gastroenterol* **104**, 465-483; quiz 464, 484 (2009).
106. Mahid, S.S., Minor, K.S., Soto, R.E., Hornung, C.A. & Galandiuk, S. Smoking and inflammatory bowel disease: a meta-analysis. *Mayo Clin Proc* **81**, 1462-1471 (2006).
107. Xavier, R.J. & Podolsky, D.K. Unravelling the pathogenesis of inflammatory bowel disease. *Nature* **448**, 427-434 (2007).
108. Rutgeerts, P., et al. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med* **353**, 2462-2476 (2005).
109. Reinisch, W., *et al*. Adalimumab for induction of clinical remission in moderately to severely active ulcerative colitis: results of a randomised controlled trial. *Gut* **60**, 780-787 (2011).